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A80
UNDERSTANDING THE MECHANISM OF SILVER NANOPARTICLE TOXICITY

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Along with the development of silver nanoparticles (AgNP) applications, the concern about their possible toxicity has increasingly gained attention. As the respiratory system in one of the main route of exposure, the aim of this study was to evaluate the harmful effects developed in lung after an acute AgNP exposure using 2D and 3D *in vitro* and *in vivo* models.When AgNP were characterized they showed a hydrodynamic diameter of 17±6 nm and they were able to initiate the hemolytic cleavage of H_2O_2 that may lead to $OH^{\bullet}$ production. First, an *in vitro* approach was done in A549 cells exposed to 2.5 µg/mL AgNP up to 24 h. A decreased in mitochondrial respiration ($p<0.01$) and in the extracellular acidification rate under stressed conditions ($p<0.05$) were observed after 3h of exposure, while a 72% increase in H_2O_2 production was observed after 1h ($p<0.001$). Moreover, increased expression of HO-1 was observed after 24 h exposure (49% $p<0.01$). In an EpiAir way 3D tissue exposed to 2.5 µg/ml AgNP for 24 h we observed a decreased in the transepithelial electrical resistance ($p<0.05$).In the *in vivo* studies, Balb/c mice (25g) were intranasally instilled (0.1 mg AgNP/kg body weight). Biodistribution was evaluated by labelling AgNP with ^{99m}Tc showing the lung as the main organ of AgNP deposition. Samples were collected 1 h after the exposure to measure lung O_2 metabolism. Tissue O_2 consumption increased by 31% ($p<0.05$), due to an increased mitochondrial active respiration (55%, $p<0.001$). Moreover, mitochondrial H_2O_2 production rate was also increased by 39% ($p<0.05$) along withan increased SOD and CAT activity (68%, $p<0.01$; 18% $p<0.01$ respectively) and a decreased GSH/GSSG ratio. Taken together, these results show that AgNP remain in the lung, may lead to damage and impaired lung function due to O_2 metabolism alterations.

A81
COMPARATIVE STUDY OF PROTEOLYTIC ACTIVITIES OF VENOM FROM ADULT AND JUVENILE SPECIES OF *Bothrops alternatus*

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Because of the technological advance in proteomic essays, ontogenic variations studies about snake venoms have become more relevant in the last years. The differences would be associated with differences in the snake venom protein composition and could have importance in the selection of the samples for production and control of quality of antivenoms. On the other hand, some authors suggested the use of venom from young snakes (yV) in order to purify some of the main components present in major proportions than in venom from adult snakes (aV). In this study, we compared venoms from *Bothrops alternatus* (yarará grande) from both stages of ontogeny, focusing on the study of the protein bands by SDS-PAGE and also on the study of proteolytic activities (metalloproteinases and serineproteases enzymes) exhibited by these venoms as the main responsible for the physiopathological action exhibited by this animal. Proteolytic activity was assessing by azocasein method, coagulant activity by citrated plasma and then, amidolyticactitivty was evaluated on BApNA. The results showed differences not only in the protein profile, but also in the enzymatic activities from young and adult snake venoms. Young snake venom coagulated the citrated plasma in the half time (6.3 s) the adult snake venom did. The relation yV/aV of proteolytic activity assessed on azocasein was 2 and 23 in the case of amydolitc action. These results highlight the differences in metalloproteinases concentration (responsible of caseinolytic activity) and especially in the serineproteinases composition (tested on BApNA), which would express in low quantities in adult snake venoms. These evidences warn about the necessity to assay the level of protection that the standart antivenom produces against a potential snakebite by young species and, at the same time, look for the development of antivenoms with pools including young and adult organisms.

A82
FLUOXETINE EFFECT ON LIFE CYCLE AND SOME MORPHOLOGICAL PARAMETERS OF *Dermestes maculatus* (COLEOPTERA: DERMESTIDAE)

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The study of cadaveric insects for detecting xenobiotics in a qualitative and quantitative way is called forensic entomotoxicology. It also studies how xenobiotics can affect insect development. Generally, antidepressants are the most chemical agents used in suicide. In a previous study was determinate and quantified fluoxetine (selective inhibitor serotonine recapture, SSRI) in *Dermestes maculatus* (Coleoptera: Dermestidae), an insect of forensic interest. In this study was evaluated the effect of fluoxetine on *D. maculatus* development. For that, adults of this species were put inside plastic containers and fed with a mixture of pig muscle and fluoxetine

(treatment) considering a 2000 mg/kg concentration to emulate lethal overdose for humans and animals. As control were used beetles fed only with pig muscle. The containers were maintained in an incubator at 30°C±1°C, 55.4% relative humidity and 12:12 h Light:Dark photoperiod. Observations and quantifications were performed diary from the beginning until ending life cycle of beetles. Larvae of each instar, pupae and adults were collected to register total body length, and in the case of larvae, were also registered cephalic width and weight. Durations of each larval instar, each stage of development and total cycle were registered. Two replicates were performed. Larval mortality was not affected by treatment, no differences were found between control and treatment. It was not found a notorious and systematic difference in the duration of egg stage that suggested a treatment effect. From larval instar 2 to larval instar 5 (L2-L5) was observed a systematic reduction of larval instar duration due to treatment. The durations of the larval stage and pupal stage were slightly short in treatment than in control. The total duration of the life cycle was almost the same in the treatment and control. When larval weight was analyzed it was not observed a systematic difference between control and treatment but was observed a systematic increase in larval length due to treatment. With respect to cephalic width, it could not be discarded an effect of treatment and if this is the case, the cephalic width would be major particularly in the last instars. The results would suggest that fluoxetine could produce alterations on *D. maculatus* development, in duration and some morphological aspects, although the total duration of the life cycle would not be altered. Because the results are based in two replicates or trials, the results will be verified with a third replicate that is being performed.

ANIMAL AND PLANT BIOLOGY AND BIOTECHNOLOGY III

A83
Senecio grisebachii BAKER (ASTERACEAE) LEAF EXTRACT: EFFECTS ON THE PEROXIDATION OF MICROSOME MEMBRANES FROM RAT LIVER

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Senecio grisebachii Baker ("margarita de campo", Asteraceae, SG) is a 20-80 cm height bush with serrated leaves and yellow daisy-like flowers. Pyrrolizidine hepatotoxic alkaloids (PAs) have been identified in the plant and have caused poisoning when eaten by cattle. Despite this, many *Senecio* spp. are used by native people from Latin America as grounded leaves or flowers mixed with creams or as decoctions to heal skin injuries. Considering the latter, a methanolic extract from dried leaves of SG was studied for its phytochemistry and peroxidation activity on microsome membranes of hepatocytes from Wistar AH/HOK rats. A series of tests were performed on the extract to determine flavonoids, phenolic compound, lipids, carbohydrates and alkaloids. PAs were detected by a field test with Erhlich reagent and characterized by thin layer chromatography (TLC). Chemiluminescence and peroxidation were initiated by adding ascorbate to microsomes. Microsomal protein (1 mg) with the addition of SG extract (0.05, 0.10, 0.20, 0.40, 0.80 and 1.6 mg) was incubated at 37 °C for 180 minutes in 0.01 M phosphate buffer pH 7.4 and 0.4 mM ascorbate. Controls with ascorbate but without extract were used. Phytochemistry demonstrated that flavonoids and phenolic compounds were the more concentrated, while TLC demonstrated the presence of one purple spot with similar Rf to retrorsine standard. Regarding peroxidation, counts per minute (cpm) originated from the light emission from microsomes incubated with SG was statistically lower (concentration dependent) when compared to controls, demonstrating protection against peroxidation. Some *Senecio* species are toxic when ingested by both humans and animals due to liver bioactivation of PAs such as retrorsine, which is present in our SG methanolic extract. However, when applied to the skin, the phenolic and flavonoids present in the leaves exert antioxidant properties, this may explain the folk use of some *Senecio* species.

A84
IMPROVEMENT OF SOYBEAN NODULATION BY BACTERIAL EXPOSURE TO NANOPARTICLES

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In the last years, there was a growing interest in the use of nanotechnology products to improve crop production. Since *Rhizobium*-legume symbiosis is a relevant system in agriculture, the goal of this work was to carry out basic studies to analyze the effects of magnetite nanoparticles (NPs) on soybean-*Bradyrhizobium japonicum* symbiotic association. Soybean seeds were inoculated with *B. japonicum* cells (Control: C) or with *B japonicum* cells exposed during multiplication to 10 ppm of magnetite NPs (BANP). *B. japonicum* used for inoculation was grown for 5 days in a rotary shaker with or without NPs. CFU were adjusted before inoculation. Seeds were left in contact with bacterial suspensions (C or BANP) for 12 hours. Soybean plants were grown on soil in a growth chamber, with periodic water irrigation, and harvested 20 or 30 days later. Bacterial NPs pretreatment improved the germination rate. Additionally, root and aerial part length and total biomass were significantly greater in BANP plants. In the same way, root surface